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Protein Turnover in Trained Male Endurance Runners Following Exercise

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Protein Turnover in Trained Male Endurance Runners Following Exercise

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Chapter I

Introduction

Protein utilization in response to exercise remains an area of interest. Supporting protein utilization following exercise to promote muscle repair continues to be a focus of research directed at optimizing nutrition interventions during the recovery period. Protein turnover is an ongoing process comprised of protein synthesis (PS) and protein breakdown (PB). Net protein balance (NET) is the difference between these two processes [NET = PS – PB]. When PS > PB, a positive NET exists and, conversely, when PB > PS NET is negative. A positive NET reflects an anabolic state and a negative NET is associated with a catabolic situation.

Nitrogen balance, a traditional method for evaluating whole body protein utilization, continues to be used to assess protein use by the body. A relatively simple method, nitrogen balance is determined from the difference between nitrogen intake and nitrogen excretion and the results evaluated similar to NET in that a positive or negative nitrogen balance is associated with an anabolic or catabolic condition, respectively.

Positive NET or nitrogen balance is important for proper growth and development of children, those recovering from disease or injury and pregnant women [37]. Negative balance is observed during some illnesses and disease and often when energy balance is negative and weight loss occurs. With specific regard to exercise, it is widely accepted that athletes who strength or endurance train have higher protein needs then their less active counterparts [60]. Resistance

exercise damages muscle tissue and proteins stimulating repair and muscle hypertrophy. Studies have shown that resistance trained athletes require protein ingestion following exercise to achieve a positive balance for optimal muscle growth [21, 51]. However fewer studies have focused on changes in protein turnover in endurance runners following an exercise bout. While the goal of endurance exercise is not muscle hypertrophy, the increase in protein oxidation and muscle damage that occurs with endurance training warrants the need to consider protein consumption during recovery [60].

The benefit of protein as a recovery supplement is the increase in the endogenous amino acid pool that results. This will have two effects; increased protein synthesis and a decrease in protein breakdown if the protein pool is a limiting factor in these processes. Essential amino acids are necessary for a positive net balance because they are not produced endogenously [10]. The branched chain amino acid, leucine is of particular importance because of it's ability to promote protein synthesis following exercise through enhancing synthetic pathways [12, 45]. Leucine exerts its effects on short-term translational controls of protein synthesis and the translation effects are synergistic with insulin [34]. Leucine can stimulate metabolic pathways to promote synthesis and decrease breakdown [45] through intracellular signaling as a regulatory factor in the insulin/ phosphatidylinositol-3 kinase (PI3-K) signal cascade to enhance protein synthesis at the level of peptide initiation following the activation by insulin [34]. Studies have shown that feeding leucine soon after exercise stimulates recovery by enhancing protein synthesis via regulation of translation [34].

While a positive NET resulting from protein supplementation following resistance training has been shown to be effective in muscle protein synthesis and repair, less is known about the specific role that leucine plays in recovery from endurance exercise. Therefore, due to its leucine content, the primary objective of the current study was to examine the effect of beef jerky consumption as a recovery snack after endurance exercise on whole body protein utilization in endurance trained males. The present study used contemporary (stable isotope methodology) and traditional (nitrogen balance) methods of protein assessment to evaluate the effects of this recovery supplement on protein utilization following endurance training. We hypothesized that beef jerky alone or in combination with a standard recovery beverage - given its high leucine content - will be associated with higher rates of whole body protein synthesis than that noted for a standard sports recovery beverage.

Chapter II

Overview of the Literature

Protein utilization can be affected by various factors. Routine exercise can increase the need for additional protein given the associated increase in protein turnover. This chapter will consider protein turnover as well as how the macronutrient composition of the diet influences the turnover and balance of protein in the body. The role of specific amino acids on protein synthesis and degradation ultimately impacting nitrogen balance will also be highlighted. In addition, traditional and contemporary assessment techniques will be presented but limited in scope to those utilized in the present investigation. Nitrogen balance represents a traditional method for assessing basic conclusions concerning protein utilization. Modern methods utilize stable isotope tracers such ^{15}N-glycine for more specific determinations of protein utilization (i.e. rates of synthesis and breakdown). These methods are relatively straightforward and noninvasive making them suitable for all populations.

Protein Turnover

Protein metabolism signifies all reactions involved in synthesis of protein, amino acid production, and the breakdown of protein and individual amino acids. Protein turnover is described as the "renewal or replacement" [70] of proteins in the body which occur through the formation of new protein or synthesis from the free amino acid pool, as well as the degradation of protein already contained in the body including various tissues, enzymes and transporters. The constant

regeneration and degradation of protein replaces old or damaged proteins with newly synthesized ones providing a means for the body to repair itself.

Protein turnover is an ongoing process comprised of protein synthesis (PS) and protein breakdown (PB). Net protein balance (NET) is the difference between these two processes [NET = PS – PB]. When PS > PB, a positive NET exists and, conversely, when PB > PS NET is negative. A positive NET reflects an anabolic state and a negative NET is associated with a catabolic situation.

Availability of amino acids as substrate affects the rate of protein synthesis [68]. Following endurance exercise in the fasted state, net whole body protein balance will be negative, however when amino acids are ingested either alone or in conjunction with carbohydrate, a shift to a positive balance occurs [7, 54, 73]. Protein ingestion following exercise increases anabolism but not all amino acids are equal in their effect on protein synthesis. Essential amino acids, more specifically leucine, have been shown to maximize the anabolic state following exercise. Bohe et al. [7] demonstrated the significance of extracellular essential amino acids following exercise in stimulating protein synthesis [66]. Leucine is considered most important for anabolic signaling [7] because it stimulates translation initiation pathways [66].

Protein and essential amino acid consumption following exercise is important for the repair of damaged and the synthesis of new proteins. Turnover represents the endogenous movement of amino acids in the body and is related to protein utilization and nitrogen balance. Changes in protein turnover are often reflected by changes in nitrogen balance.

Nitrogen Balance

Nitrogen balance has long been considered representative of the connection between the availability of amino acids for protein synthesis and the breakdown of endogenous protein. Nitrogen differentiates protein from the other macronutrients, carbohydrate and fat. Therefore, changes in nitrogen use by the body provide insight regarding protein utilization. When dietary nitrogen intake (N_I) equals the output in the urine, feces and sweat (N_O), nitrogen balance occurs. This reflects a state of equilibrium or maintenance and is considered the minimal amount of dietary protein required for adequate protein synthesis and breakdown [37]. Positive nitrogen balance, when $N_I > N_O$, is a situation associated with tissue growth and represents an anabolic state. When $N_O > N_I$, negative nitrogen balance results subsequent to protein breakdown and the likely use of amino acids as a source of energy through Krebs cycle intermediates or gluconeogenesis in times of starvation, [37]. This is considered a catabolic state.

Available free amino acids for protein synthesis come from dietary protein as well as the endogenous production of non-essential amino acids from available nitrogen and carbon skeletons. Amino acid losses also known as nitrogen losses can occur in two forms, obligatory and oxidative. Obligatory losses occur from growth and maintenance of regulatory processes in the body and maintain homeostasis of protein and amino acids even when no protein is being consumed [39]. Oxidative losses include regulatory losses occurring from changes in physical activity as well as changes in protein intake [39]. Because nitrogen balance does not provide specific insight into changes in protein

synthesis or protein breakdown by the body, stable isotope techniques are used to better characterize the components of whole body protein turnover.

Protein Assessment Methodology

Nitrogen Balance. Nitrogen balance is a noninvasive method considered to be the standard for defining minimum levels of dietary protein required for various populations. This method determines the variation between dietary protein intake, which contains 16% nitrogen, and the amount of nitrogen lost through excretion. Being that protein is the only macronutrient that contains nitrogen, the difference between nitrogen intake from the diet, and nitrogen output from excretion factors (urine nitrogen, fecal losses, etc.), yields net nitrogen, or protein, balance.

Advantages/Disadvantages. Advantages of the nitrogen balance method are its simplicity and usefulness for all populations. This method presents an affordable way to determine protein needs as well as protein status in various populations. The primary disadvantage of this method, however, is that it provides no information concerning specific movement in protein pools within the body. With this method alone, the contributions of both protein synthesis and protein breakdown to net turnover cannot be determined.

Limitations. Limitations of nitrogen balance methodology include dietary protein intake levels, completeness of urine collection, and accuracy of dietary records used to determine nitrogen intake. Problems arise when protein intakes are too low or too high because the body adapts to these diets resulting in nitrogen retention or an impractical positive balance. Energy intake should not be

over looked in addition to protein intake because in low energy situations, protein will be used as a source of energy resulting in a more negative nitrogen balance when energy needs are not met. Given that nitrogen excretion is determined from urine output, it is important that urine collections be complete to have accurate analysis. Finally, accurate diet records are important for determination of nitrogen intake. If diet records are not accurate, protein, and therefore nitrogen, intake cannot be accurately determined resulting in inaccurate measures. Even so, nitrogen balance remains the standard of reference for determining protein and amino acid requirements in all populations.

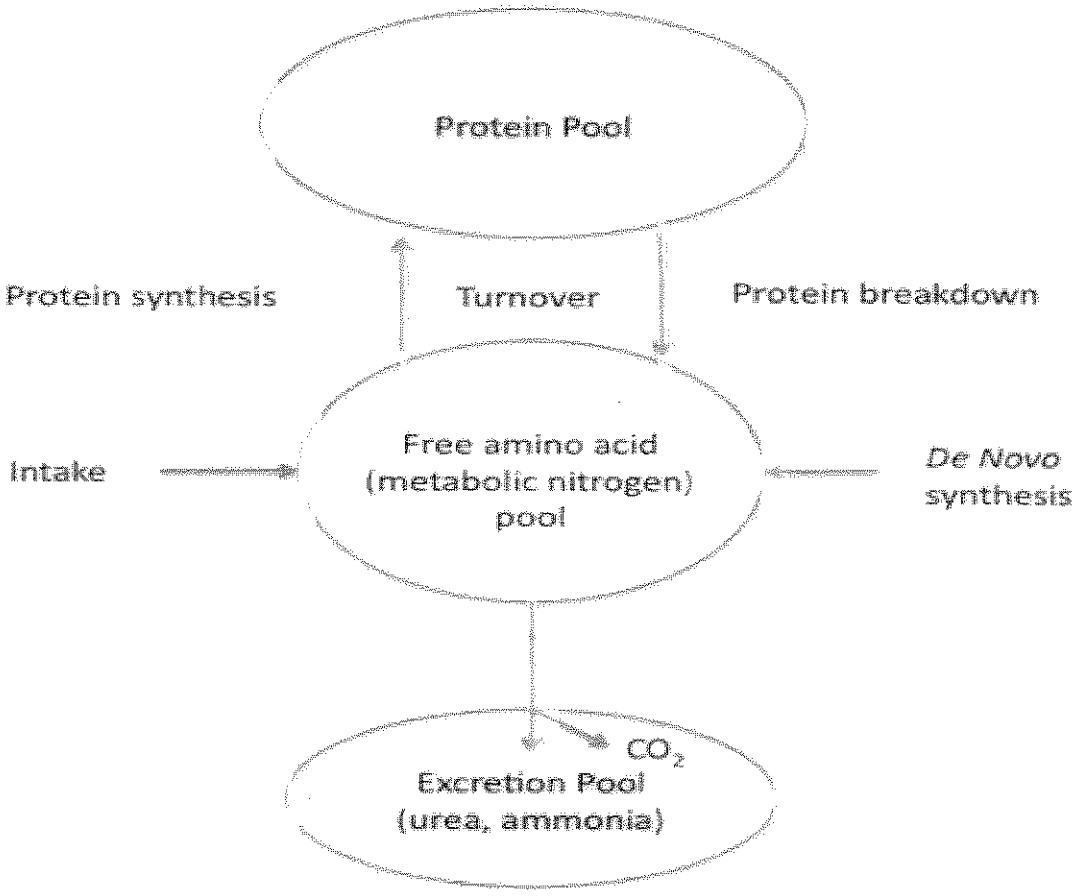
Stable Isotope Methodology

Stable isotope methodology has been used to characterize protein turnover in humans. Common isotopes used for these techniques include those with labeled nitrogen (i.e, ^{15}N-glycine) or carbon (i.e., ^{13}C-leucine). This thesis utilized tracer methodology specific to ^{15}N-glycine or the end product method. There are four assumptions of the model for this tracer technique: 1) the three-pool model is fundamentally correct and the isotope is spread throughout each pool, 2) protein synthesis and excretion are the primary means of removing nitrogen from the free amino acid pool, 3) ingested ^{15}N-glycine is metabolized in the same manner as endogenous and exogenous amino nitrogen, 4) there is no significant amount of recycling of isotope [2].

The main assumption of ^{15}N-glycine end product method involves a three-pool model which represents a free amino acid pool, protein pool, and excretion pool [20] for the determination of protein synthesis and breakdown, as well as flux.

Figure 2.1 illustrates the three-pool model used in assessing whole body protein turnover. The free amino acid pool holds amino acids from dietary protein, the degradation of endogenous protein and those nonessential amino acids that are synthesized in the body. The protein pool contains all protein synthesized in the body and the excretion pool is the broken down protein and amino acids in nitrogen form that is excreted.

Figure 2.1. Three-pool Model.



^{15}N-Glycine. The choice of glycine over other amino acids is due to its ability to be evenly distributed between the end product pool and the protein pool as well as the consistency of the results derived between dosage given orally as well as intravenously [70]. Glycine also satisfies the basic tracer assumption that the amount of tracer going to the end product and to protein is equally proportional to the amino-N flux going down these two pathways [26, 70].

The end product method is based on the assumption that the two end products (protein synthesis and excretion) from the free amino acid pool are equal assuming that the free amino acid pool is the only source for the products [20]. It is then assumed that the excretion is equal to the amino nitrogen mixture that enters the protein pool or also stated as the proportions of flux that go to synthesis and excretion are the same proportions of tracer that go to synthesis and excretion [20]. The amount of nitrogen excreted is considered to be the total nitrogen excretion when only collecting urine because other forms are considered negligible or can be factored in later [20]. Fundamentally, protein synthesis and excretion have to be equal or the calculation of nitrogen excreted as the end product would be an invalid measure of protein synthesis.

^{15}N-glycine can be given either orally or intravenously with small differences found between the two routes. In this investigation the ^{15}N-glycine was administered orally in one large single dose (2mg/kg body weight) under fasted conditions followed by urine output collection for the next 9-10 hours. Oral administration is noninvasive, easier, and more convenient for the subject and is suitable for population studies for testing whole body protein turnover [69].

Assumptions of this method and also considered disadvantages to oral administration are that it requires normal gut function, the amino acid is not metabolized in the gut before it can enter the blood stream and the liver does not take up the amino acid in the first pass [2]. Although either route is acceptable, oral administration is preferred because its whole body protein metabolism values tend to be slightly higher than the intravenous route [2]. Nutritional status during the study must be taken into consideration because ingestion of glycine in the fasted state can decrease synthesis values by 20% [20].

Ammonia was chosen as the end product to measure flux of protein turnover because it turns over quicker than urea and can be determined with a urine collection of only nine to ten hours to eliminate possible complications such as an incomplete labeling if too short or a recycling of label if too long [20]. Although urea can be used as the end product, it is generally found that it gives an inflated synthesis rate, and it is important to note that the two methods do not correlate [2]. Although not proven, this lack of relationship is hypothesized to be due to the production of urea in the liver having a bias toward hepatic protein metabolism whereas ammonia is produced in the kidney and is biased toward muscle protein metabolism [2].

Muscle Damage During Exercise

Exercise causes physical damage in the body from unfamiliar stresses, eccentric contractions, and reactive oxygen species (ROS) from elevated breathing. Damage occurs through disruption of fibers which are linked to inflammatory responses and changes in excitation-contraction coupling in muscle

[17]. It has been suggested by Friden et al. that the z-lines in muscle fibers which are linked by cytoskeletal protein desmin and part of the myofibrilar chain could be the site of muscle damage due to its susceptibility to exercise induced disruption. [24].

More specifically, endurance runners can experience delayed onset muscle soreness following endurance events inducing inflammation [62]. An increase in neutraphil mobilization [27] is correlated with the percent VO_{2max} of a given exercise bout [57] and has been shown to increase ROS and oxidative damage to tissues following endurance exercise [55]. Damage can also be assessed by the appearance of muscle proteins in the blood particularly creatine kinase which is an indirect marker of muscle damage [17]. The damage occurring during exercise requires repair, which is helped by the availability of nutrients, in particular protein and carbohydrate [41].

Effects of Habitual Training on Protein Needs

As exercise training continues the body adapts to the stimulus and increased stimuli is required for further physiological adaptations. Chronic training through either resistance or endurance exercise can have profound impacts on protein metabolism and fundamental adaptations in muscle. Adaptations to resistance training include muscle hypertrophy while endurance exercise increases oxidative capacity [67]. Training has mixed efects on protein turnover. Pikosky et al. [50] showed an increase in protein breakdown and an even larger increase in protein synthesis which resulted in a positive net protein balance in muscle following 4 weeks of aerobic training in unfit subjects. Carraro

et al. [16] demonstrated after four hours exercise at 40% VO_{2max} protein breakdown increased with an even more significant increase in fractional synthetic rate during the recovery period in the exercised group compared to the control group. On the other hand following a combination of aerobic and resistance training, protein breakdown was unchanged whereas synthesis increased [65].

Relative to work done with resistance training, protein turnover in response to habitual or acute endurance exercise is less studied [4, 5, 47, 48, 51, 64]. With strength training, the body adapts to the training stimulus such that there is an increase in nutrient needs for further exercise adaptations and gains in lean muscle. Relative protein consumption in particular must increase to achieve positive nitrogen balance for increased protein synthesis to ensure adequate muscle recovery and growth to increase muscle mass [67].

The lack of information regarding the extent endurance exercise adaptations specific to protein metabolism is a limitation of this review. Still, it can be inferred that endurance exercise has a significant impact on protein metabolism. Aerobic exercise increases the mitochondrial enzyme activity as well as size and amount of mitochondria in muscle [44] indicating metabolism for at least some amino acids should be increased due to aerobic training [67]. The effects of endurance exercise on protein utilization have been reported to last from a few minutes to a few days although reports of exact length and effect on metabolism differ [68]. Following endurance exercise, no change [19, 65] or a decrease [52] in whole body protein breakdown have been demonstrated. Whole

body protein synthesis on the other hand increases following endurance exercise [19, 52]. Leucine oxidation increases as well during situations associated with increased protein degradation including increased exercise intensity and duration [68].

As indicated by leucine appearance, protein breakdown can increase during exercise [15, 52] resulting from the use of leucine as a source of energy for gluconeogenesis and more available amino acids. However, this does not always equate into increased urea production during or following exercise [15] possibly due to an acute phase plasma protein synthesis during and following exercise [14, 15]. In addition, the exercise induced inflammatory response and the subsequent series of reactions associated with this condition increases rates of protein turnover necessary for repair [39]. Protein turnover can be affected by amino acid availability following exercise as protein consumption contributes to an increase in protein synthesis and a decrease in protein breakdown during recovery from the exercise bout [36].

To show the importance of protein/amino acid availability to recovery from exercise many studies use supplements where carbohydrate amount is matched so any differences in results will be due to protein only. This design allows for the effects of carbohydrate on protein turnover to be observed as well. Howarth et al. [31] compared the effects of a protein-carbohydrate (PRO-CHO) mixed drink against a low (L-CHO) and high (H-CHO) carbohydrate drink following 2 hours of cycling exercise. The L-CHO drink matched the amount of carbohydrates in the PRO-CHO mixture while the H-CHO drink matched the PRO-CHO drink in

calories. Immediately after and 4 hours following exercise, fractional synthetic rate (i.e. rate of synthesis over a given time) was higher in PRO-CHO than either the L-CHO or H-CHO. In addition whole body nitrogen balance was positive only in the PRO-CHO trial, which was mainly attributed to a decreased rate of protein breakdown.

A second comparison between overall calorie consumption (CHO only drink) and protein content (isocaloric CHO+PRO drink) on whole body protein turnover was performed by Murphy and Miller [42]. Healthy older adults with an average age of 59 exercised on 2 separate occasions for 1 hr at 50% VO_{2max} followed by a 4 hour rest period where either a protein-carbohydrate (PRO) drink (40g carbohydrate, 20g whey protein) or an isocaloric carbohydrate (CHO) drink (60g carbohydrate) was consumed. Results showed a significant increase in circulating essential amino acids, non-essential amino acids, and leucine in the PRO group but these values were not significantly different at the end of the 4-hour recovery period. Results also showed an increase in leucine rate of appearance (Ra) and rate of oxidation (Rox) from 2 to 4 h post-exercise in the PRO drink as well as greater rates of non-oxidative leucine disposal. Given these results the main finding was an overall increase in whole body protein turnover during recovery when a protein-carbohydrate drink is consumed compared to a carbohydrate only drink.

In a study comparing fat free chocolate milk (MILK) and a carbohydrate (CHO) beverage following recovery of an endurance run, Lunn et al. [36] found a 38% higher fractional synthetic rate as well as a 5% decrease in leucine Ra

which is a sign of protein breakdown. The beverages were isocaloric and neither contained fat but the MILK drink contained 16g of protein and the CHO drink contained 16g more carbohydrate. In context of a controlled diet and a consistent training routine, the study found increased markers of muscle protein synthesis as well as a decrease in whole body protein breakdown following consumption of a MILK drink.

When supplements are matched in one macronutrient they are usually not isocaloric, which can affect recovery if the calorie difference is large. As a result, some studies design supplements to be isocaloric but neglect the one macronutrient that is being researched. Levenhagen et al. [35] showed increased whole body protein and leg muscle protein when a combination drink of 10g protein, 8g carbohydrate, and 3 g fat (SUPP+PRO) were ingested instead of an isocaloric carbohydrate and fat drink (SUPP) or a no nutrient drink (NO). After cycling for 60 minutes at 60% VO_{2max} subjects were given one of the 3 recovery drinks at random and were tested for a number of factors including individual amino acid concentrations, net amino acid balance across the leg, and whole body protein measurements. Results of amino acids show SUPP+PRO increased plasma branched-chain amino acids (BCAA) 47% and 53% over NO and SUPP respectively as well as total amino acids 21% and 14% over NO and SUPP respectively. Net amino acid balance in the leg also produced a net uptake compared to the net release in the NO and SUPP groups. Using leucine Ra, whole body proteolysis was unaffected by the different drinks. The SUPP+PRO group tended to increase protein synthesis 15% over the NO group but showed

little difference compared to the SUPP group. Overall the SUPP+PRO drink stimulated increased circulation and uptake of amino acids, and enhanced leg protein synthesis. In addition, net leg and whole body protein accretion when protein was included had a direct relationship to the net losses seen when no nutrients or only carbohydrate and fat were provided.

Recognizing the beneficial effects of leucine, Nelson et al. [43] tested the effects of a leucine enriched protein-carbohydrate-fat (LEUPRO) drink compared to an isocaloric carbohydrate-fat only (CON) drink for recovery after 6 days of aerobic cycling training blocks. Subjects consumed isocaloric standard diets with CON consuming 1.5g/kg/d protein and LEUPRO consuming 1.9g/kg/d inclusive of supplements so the difference between the diets was due only to the protein consumed during recovery. After the cycling period for that day the subjects were given the drink and went through a battery of tests including whole body leucine turnover, muscle damage, and protein turnover during a recovery period that matched the length of the workout for that day. Overall results showed LEUPRO increased non-oxidative leucine disposal resulting in positive leucine and nitrogen balance. Creatine kinase excretion was also decreased in LEUPRO during the 6 day training block suggesting a decrease in tissue breakdown. During days 2-5, nitrogen balance was positive in both conditions however LEUPRO increased recovery plasma amino acids and secured net positive whole-body leucine balance. Overall LEUPRO should have made up for whole-body protein oxidative losses and supported the 50% increase in protein synthesis thus resulting in the highest rate of protein retention and optimizing

muscle synthesis. In total, aforementioned studies demonstrate a positive effect of protein, as well as leucine, consumption to protein utilization during recovery from endurance exercise.

Energy and Nutrients for Exercise and Recovery

Energy. Caloric intake is important to provide energy for the body to function. When completely at rest, the body requires a certain amount of calories for basic cell function and any calories above this amount are used for locomotion. Physical activity represents normal daily muscle contraction while exercise includes all forms of planned muscular, physical and structured movement of the body designed to enhance physical performance [25]. Adequate energy intake is defined as the amount of calories needed to balance total energy expenditure and maintain body weight. Calorie intakes in excess of energy expenditure on a daily basis will lead to weight gain usually in the form of fat although a small amount of excess energy is required for muscle growth during weight training. Calorie restriction will lead to weight loss from lean mass and fat mass.

Athletes commonly use calorie restricted diets to make weight classes, to lose body fat, to improve skill related fitness, and for aesthetic reasons. Calorie restrictions however can lead to impaired performance when restricted up to 7 days [25] in which case decreased performance could be due to dehydration or a lack of glycogen stores [29]. Lack of sufficient calories can decrease glycogen and glucose levels resulting in decreased time to exhaustion in endurance athletes [33] and body weight may fall below levels required to sustain physical fitness [25]. In

addition chronic inadequate energy ingestion can lead to a deficiency of certain nutrients that may be integral to performance through optimization of metabolic function [53]. While training, athletes need to consume enough calories to maintain weight and body composition [61] as a reduction in weight can compromise performance and negate training benefits [53]. However, studies have shown that weight loss in relation to fat mass has no effect on strength or muscular endurance in normal and overweight subjects [25].

In addition to decreased glycogen stores from reduced caloric intake, signals in the protein breakdown pathway may be attenuated by calorie intake post-exercise [28] Harber et al. studied the fractional synthetic rate (FSR) and the expression of genes involved in muscle remodeling following aerobic exercise in both a fasted state and a fed state. Eight male subjects cycled for 60 minutes at 72% VO_{2max} and consumed either a non-caloric placebo (EX-FAST) or a beverage containing 5 kcal/kg body weight (EX-FED) immediately and 1 hr after exercise. FSR was higher after exercise in both groups compared to their resting values but EX-FED showed attenuated mRNA expression of proteolytic markers MuRF-1 and calpain-2 but no change in expression of markers associated with muscle synthesis FOXO3A, calpain-1, caspase3 or myostatin mRNA compared to EX-FAST. This shows that although feeding does not increase synthesis, it does decrease breakdown as shown by factors involved in the ubiquitin-proteosome and Ca2+-dependent degradation pathways following non-exhaustive aerobic exercise.

Fat. The importance of fat for performance and recovery has had little attention compared to other macronutrients because it is not directly associated with muscle size (i.e. protein) or immediate energy stores (i.e. carbohydrate). While fat does not seem to have any significant physiological effect following exercise, it can be a good source of calories providing 9 calories per gram, which will benefit exercise recovery. It is the position of the American Dietetics Association (ADA) that athletes should not consume a high fat diet and that fat intake should be sufficient to provide essential fatty acids, fat soluble vitamins and provide energy for weight maintenance [53]. The Position Paper on Nutrition and Athletic Performance [53] recommends a diet for athletes that contains 20-35% of total energy intake from fat and diets having less than 20% from fat consumed in an effort to reduce body fat percentage does not benefit performance. It should be emphasized that fat is a necessary component of the diet and beyond supplying calories is an essential fuel source, is part of cell membranes and functions in the transport of fat soluble vitamins like A, D, and E [53].

Horvath et al. [30] confirmed this position with results from aerobically trained men and women on diets with varying fat intake. Male and female runners were assigned to an isocaloric low fat (16%), medium fat (31%),and high fat (44%) diet for four weeks and upon the conclusion of each trial were tested for VO_{2max} and endurance. Results showed that subjects on the low fat diet ate 19% fewer calories than the medium fat or high fat group. However, weight, percent body fat, VO_{2max} , and anaerobic power was not affected by dietary fat percentage. Endurance time to exhaustion was 14% higher on the medium fat

diet than the low fat diet and improved 20% in females and 8% in males. Overall, Hovarth et al. concluded that runners on a low fat diet consume fewer calories and have reduced endurance performance due to a decrease in glycogen stores. Furthermore, a medium or high fat diet did not negatively affect anaerobic power or time to exhaustion.

Carbohydrate. Carbohydrates are arguably the most important macronutrient during physical activity. Carbohydrates provide immediate energy for glycolysis during athletic events and provide a stored source of prolonged energy as glycogen. Also, upon ingestion, carbohydrates produce an insulin response that has been shown to have beneficial effects in decreasing protein breakdown as well as increasing blood flow to tissues for increased recovery [72]. Given these benefits, carbohydrates are important to promote recovery following exercise.

The amount of optimal carbohydrate ingestion varies depending on the athlete as well as the event. The recommendation is consumption of 200-300g of carbohydrate 2-4 hours before an aerobic event [22] to maintain blood glucose and maximize glycogen stores [53] which is the major substrate for sub-maximal and intermittent high intensity exercise including aerobic training and resistance training [25]. During cycling exercise carbohydrates produce an ergogenic effect by increasing time trial performance [22], time to exhaustion [38], as well as power output and self selected pacing [71]. While not all studies have concluded that carbohydrates provide added benefit during exercise, these differences in effects

could be due to the type of carbohydrate consumed, amount and timing of ingestion [25] as well as the type of exercise performed.

Carbohydrate following exercise promotes the resynthesis of glycogen. Exercise intensity and duration can influence the amount of carbohydrate necessary for glycogen repletion because events of long duration such as a marathon will deplete glycogen stores more than exercise of shorter duration. The consumption of 1-1.5g/kg carbohydrate every 2 hours for 6 hours is recommended for glycogen resynthesis starting immediately after exercise [53]. Delaying carbohydrate consumption even by 2 hours has shown to negatively affect glycogen re-synthesis [32, 72].

In response to carbohydrate ingestion, insulin is released and has been shown to be the possible reason for an increase in positive net protein balance following exercise when carbohydrate alone is consumed. Borsheim et al. [9] studied the effects of 100g of maltodextrin carbohydrate (CHO) ingestion alone against a placebo (PLA) on net muscle protein balance following resistance exercise. Following a resistance training workout of 10 sets of 8 reps at 80% 1 repetition maximum (RM) subjects laid in bed for 4 hours and consumed either CHO or PLA 1 hour following exercise. Insulin levels showed no change following the PLA but rose following CHO. Net muscle protein balance did not change in PLA but improved during the second and third hour of recovery from an average of -17 mmol/ml to -4 mmol/ml and 0 mmol/ml respectively. It appears that the increased balance was due to a decrease in breakdown, however, the use of carbohydrate alone did not produce a positive balance and the improvement was

minor compared to protein balance results seen in other studies when amino acids alone are consumed during exercise recovery.

Protein. Amino Acids are necessary building blocks for the synthesis of protein used to rebuild tissue after exercise. Protein metabolism during and following exercise can be affected by gender, age, intensity, duration, type of exercise, energy intake and carbohydrate availability [53]. Although no official guidelines exist, it has been shown that those who participate in physical exercise have an increased need for dietary protein [60] not only following exercise but have an increased daily protein requirement.

Currently the Recommended Dietary Allowance (RDA) for protein is 0.8 g/kg/d protein for the average person. However this does not take into consideration physical stress or habitual exercise/exercise training. The recommendation for strength and endurance athletes exceeds the RDA at 1.2 to 1.7 g/kg/d protein [53]. An increase in protein oxidation during endurance exercise and a decrease in nitrogen balance shown in endurance runners suggests that a higher protein intake is beneficial [11, 46, 60]. Tarnopolsky et al. [60] concluded bodybuilders need only 1.2 g/kg/d and endurance athlete need 1.6 g/kg/d for safe mixed protein intake that achieved protein balance. The researchers further concluded during habitual training bodybuilders require a daily protein intake only slightly higher than that of a sedentary individual to maintain lean body mass and endurance athletes require greater protein intakes than both bodybuilders and sedentary individuals to meet the needs of protein breakdown from exercise because of the increased muscle damage due to continuous muscle contractions,

strain from the repeated impacts, and greater reactive oxygen species perpetuating muscle damage.

Different protein levels have been suggested for athletes participating in different sports, ranging from the current 0.8 g/kg/d for light to moderate intensity exercise to 1.1-1.4 for moderate to heavy endurance and resistance training and 1.5-1.8 for elite endurance athletes [37, 59]. Although many endurance athletes already consume more protein than necessary, elite athletes may benefit from increasing their protein intake even beyond the 1.8 g/kg/d, and for this reason it could be beneficial to refine these protein needs through further research [59].

Protein intake following exercise increases protein synthesis and overall net balance [4, 5, 11, 14, 35, 42, 64]. The timing, type and amount of protein can affect the extent of synthesis and balance however [51]. An array of protein products are used by athletes most of which contain either whey, casein or soy protein, that differ based on rate of digestion. Studies comparing the three show that while all produce positive nitrogen balance, muscle protein synthesis is stimulated to a greater extent by whey and soy protein than casein [58]and in addition peak leucine is greater in whey than casein [63]. Furthermore, Moore et al. found only 20g of protein were needed to induce maximum protein synthesis following exercise [40]. The addition of carbohydrates to a protein recovery drink increases calories allowing amino acids to be used for protein synthesis instead of being used as a source of energy [54] and leading to greater exercise training adaptations [23].

Recommendations

Protein is one of the most commonly used recovery aids in the supplement market because of its effects on protein utilization following exercise. Protein is important for inducing positive nitrogen balance repairing damaged proteins. The addition of leucine or consumption of protein products with high leucine content can further stimulate the recovery process by increasing protein synthesis [34, 45] and sparing protein from breakdown [12]. Calorie contribution from carbohydrate and fat are also important for provision of energy for tissue repair allowing dietary protein to be spared and to function in an anabolic manner. Moreover, leucine and insulin act synergistically to promote protein synthesis by inhibiting the initiation 4 complex through activation of protein kinase mammalian target of rapamycin and stimulate the phosphoinositol 3-kinase signaling pathway respectively [45].

Given the anabolic effect of insulin on decreasing protein breakdown and increasing glycogen synthesis and the effect of protein intake on enhancing synthesis and decreasing breakdown it seems logical that a mixed protein-carbohydrate drink would be most beneficial to the endurance athlete. The diminished glycogen stores, increased tissue damage from continuous muscle contraction and generation of ROS following an endurance exercise bout suggest that the use of a combined supplement would promote optimal recovery from endurance exercise.

The aim of the present study was to characterize whole body protein turnover in trained runners during recovery from an endurance run following 6

days of consumption of beef jerky alone following exercise (with water only) or in combination with a traditional recovery (carbohydrate-electrolyte) beverage as a recovery snack following endurance training.

Given the high leucine content of beef jerky we hypothesized that beef jerky alone or in combination with a standard carbohydrate-electrolyte recovery drink would contribute to increased rates of whole body protein synthesis compared to consuming a traditional carbohydrate-electrolyte recovery beverage alone.

The purpose of the present investigation was to evaluate the effects of beef jerky, a carbohydrate-electrolyte beverage (here in referred to as Powerade®), or a combination of both on protein utilization in trained male runners. Specifically the study utilized the nitrogen balance and ^{15}N-glycine methods to differentiate the effects on protein utilization over three one-week training cycles in healthy trained male runners ages 18- 30 years.

Chapter III

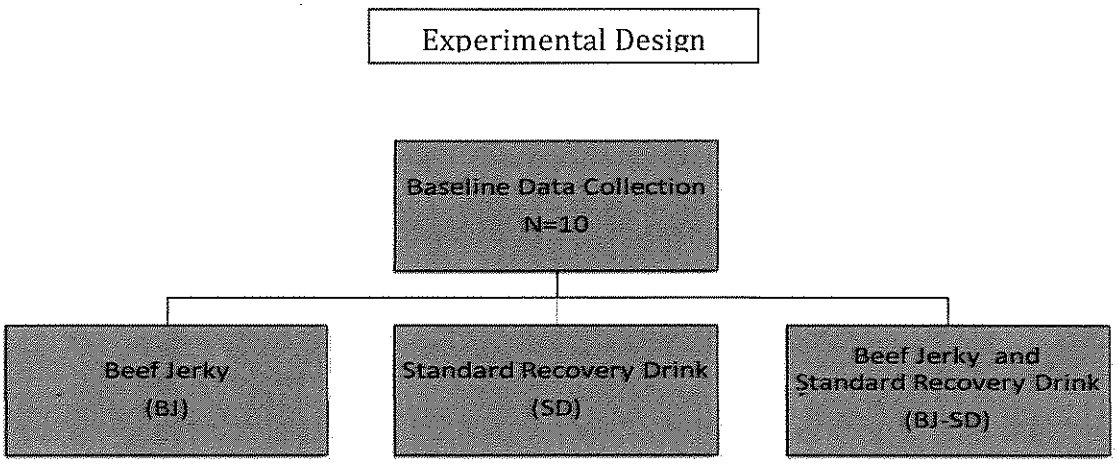
Methods

Study Design

The study design consisted of a baseline period followed by three one-week supplementation cycles. Criterion measures were assessed at baseline and at the end of each supplementation cycle. Subjects recorded all dietary intake and exercise training throughout the investigation period. The Institutional Review Board at the University of Connecticut at Storrs approved this specific protocol.

Ten healthy, endurance trained men were recruited to participate in all research protocols in a random order. Subjects consumed a eucaloric (weight maintenance) diet for which protein and fat intakes were held constant (1.5 $g \cdot kg^{-1} \cdot d^{-1}$ protein, dietary fat approximating 30% of total energy). Subjects were randomly assigned to recovery snack treatment. Following 6 days of consuming either Beef Jerky only (BJ), a standard recovery drink only (SD) or a combination of Beef Jerky and a standard recovery drink (BJ-SD) during recovery from their routine training runs, participants underwent an exercise protocol for assessment of whole body protein turnover and criterion measures specific to hydration status post exercise. Subsequently, subjects crossed over to a second recovery snack, repeated the protocol, and then crossed over to the final recovery snack assignment. Subjects were given a 2 day 'washout' period during which diet and exercise training were monitored. The study design is shown in Figure 3.1. This thesis is specific to protein turnover assessments.

Figure 3.1. Study Design



Subjects

Ten male subjects volunteered to participate in this investigation. All subjects were healthy non-smoking young men between 18 and 30 years old who had been training for at least two years, run a minimum of thirty miles per week, and had displayed a VO_{2max} > 50ml O_2/kg during a maximal oxygen consumption test documenting them as "trained" runners. Exclusion criterion included: taking medications that altered fluid balance, vegetarian, following a diet that involves consuming a high percentage of total calories from protein, and being on prescription medication for high blood pressure. Subjects completed medical history and consent forms before participation began.

Exclusion from Analysis: Two subjects were excluded from analysis after being accepted into the study. Subject CR4 experienced knee pain following cycle one and was removed from the study. All measures were taken to ensure subject safety. Subject LP8 completed the study but was removed from analysis

because he did not comply to the study protocol and therefore his values were out of the realm of the required macronutrient composition for the study.

Study Protocol

Upon being accepted into the study, subjects were tested for resting energy expenditure and maximal oxygen consumption (VO_{2max}) using indirect calorimetry. Based on VO_{2max}, 70% of max was determined for subsequent laboratory runs. Subjects were instructed to record all dietary intake and exercise on food and exercise logs, respectively throughout the study. Subjects were required to run at least 6 days a week and record heart rate using Timex heart rate monitor watches (Timex Ironman Race Trainer Heart Rate; Timex, North Little Rock, AR). On the 1st, 4th and 7th day of each cycle, subjects reported to the laboratory for their daily run. On days 1 and 4 subjects ran for 40 minutes at 70% VO_{2max} and day 7 ran for 60 minutes. VO_{2max} checks were performed at specific time intervals during the runs. Following each run during the cycles, subjects were instructed to consume the randomly assigned supplement as soon as possible at conclusion of exercise. At baseline and the end of each cycle, ^{15}N-glycine was consumed and urine was collected over a 10 hour period and a subsequent 24 hour period.

Resting Energy Expenditure (REE): REE was determined using indirect calorimetry using a Parvo metabolic cart (Parvomedics; Sandy, UT). Subjects were instructed not to consume caffeine or to exercise 24 hours before the test and do only minimal movement upon waking the morning of the test. Subjects were transported to the lab at 6 am. Upon arrival, the subject immediately lay

down and rested for 10 minutes before the test began. The test lasted for a total of 30 minutes.

Maximum Oxygen Consumption (VO_{2max}): VO_{2max} was determined through indirect calorimetry using a Parvo metabolic cart on a treadmill. Following the REE subjects were fed a standard breakfast of a plain bagel, 2 tablespoons peanut butter, banana, and water and were allowed to rest as long as they needed before performing the VO_{2max}. Before the test subjects were allowed to perform their own warm up as well as walked on the treadmill for 5 minutes. The test started at a pace determined from their normal running pace and at a 1% grade. Pace was not changed during the test and grade was increased 1% every minute. Subjects were attached to a heart rate monitor and tested for rating of perceived exertion before every increase in incline. Subjects were encouraged to run as long as they could but were instructed to stop whenever they felt they could not continue. The test lasted between 8-15 minutes. VO_{2max} is the maximum rate of oxygen consumption measured during exercise. Oxygen consumption equals amount of oxygen inspired minus oxygen expired. Oxygen inspired is calculated by multiplying the percentage of oxygen inspired by the volume of air inspired. Similarly, the amount of oxygen expired is calculated by multiplying the percentage of oxygen expired by the total volume of air expired. When using the metabolic cart, the VO_{2max} is attained from the following criteria, Respiratory Exchange Ratio (RER) is $\geq$ 1.1, maximal heart rate (HR) within 10 b/min of the calculated value, or an O_2 plateau ($\Delta VO_2 \leq 50$ mL/min) with an increase in power output.

Criterion Measures

Diet Records and Nutrient Intake. Subjects were required to keep records of all dietary intake starting at the baseline period through the completion of the study to document and monitor macronutrient, sodium and fluid intake. Records were collected at each lab visit and were checked for accuracy and completion. Diet records were analyzed using Nutritionist Pro software (Axxya Systems, Stafford, TX). Subjects were required to consume 1.5 g protein/kg body weight throughout the investigation and were instructed to increase or decrease protein consumption accordingly. The required protein consumption included protein provided from supplements. Supplement nutrient breakdown is shown in Table 3.1.

Table 3.1. Nutrient Composition of Recovery Snacks from the USDA Standard Reference Database.

Beef Jerky (1 oz)	**Powerade**	**Beef Jerky (1 oz) + 12 oz Powerade**
Kilocalories: 116		
Protein: 9 g	*Serving Amount: 12 oz Powerade*	Kilocalories: 233
Carbohydrate: 3 g	Kilocalories: 117	Protein: 9 g
Fat, Total: 7 g	**Protein/leucine: 0**	Carbohydrate: 32 g
Cholesterol: 14 mg	Carbohydrate: 29	Fat, Total: 7 g
Saturated Fat: 3	Fat, Total: 0	Cholesterol: 14 mg
Sodium: 627 mg	**Sodium: 80 mg**	Saturated Fat: 3.106
Potassium: 169 mg	**Potassium: 66 mg**	**Sodium: 708 mg**
Leucine: 895 mg		**Potassium: 217 mg**
Isoleucine: 512 mg		**Leucine: 895 mg**
Lysine: 935 mg		Isoleucine: 512 mg
Methionine: 285 mg		Lysine: 935 mg
Phenylalanine: 448 mg		Methionine: 285 mg
Valine: 551 mg		Phenylalanine: 448 mg
Histidine: 387 mg		Valine: 551 mg
		Histidine: 387 mg

Protein Turnover. Whole body protein turnover was assessed using the single-pulse stable isotope method of ^{15}N-glycine and end product analysis. This method was convenient and non-invasive, posing no threat to the subjects. Administration of ^{15}N-glycine was done on the final day of each cycle. Upon leaving the lab, subjects were given the respective ^{15}N-glycine dose in a sterile container, a spot urine cup, a 10hr urine collection jug, 2-24hr urine collection jugs, and a juice box. Immediately preceding administration of the isotope a spot urine sample was collected for determination of background ^{15}N-ammonia enrichments. Subjects were instructed to consume the ^{15}N-glycine (2mg/kg body weight; 98+ atom % enrichment; Cambridge Isotope Laboratories, Andover, MA) dissolved in fruit juice, at least 2hrs after finishing dinner. For approximately 10 hrs after the dose subjects were instructed to collect all urine in the 10hr collection jug and were instructed to refrain from exercise and consuming any food or beverage. Subsequently, urine was collected in the 24hr collection jugs for approximately 24hrs. Urine was collected in provided containers that contained 15 mL of 30% hydrochloric acid to preserve urinary ammonia.

Urinary nitrogen excretion (E) during the 10hr collection period was determined in duplicate using the micro-kjeldahl technique (Tecator Kjeltec Systems, Hogans, Sweden). The ^{15}N-ammonia enrichment was determined using isotope ratio mass spectrometry (IRMS) (Metabolic Solutions, Merrimack, NH). Subsequently, these calculations were used to determine the t:t ratio (i.e. ratio of tracer;tracee) which was then corrected for background ^{15}N-ammonia enrichment.

The ^{15}N-ammonia enrichment data was used to calculate Nitrogen Flux (Q), protein synthesis (PS), protein breakdown (PB) and net protein turnover (NET) were calculated using the following formulation, where d is the oral dose of ^{15}N-glycine (d=g glycine x 0.1972) [56].

Q [g N/(kg · d)] = [d/(corrected t:t)/10 hr · 24 hr/body weight]

PS [g/(kg · d)] = [Q – (E/10 hr · 24 hr/body weight)] · 6.25 g protein/g N

PB [g/(kg · d)] = [Q – (I/10 hr · 24 hr/body weight)] · 6.25 g protein/g N

NET [g/(kg · d)] = PS - PB

Nitrogen Balance. A 24 hr urine collection was performed and 24 hr nitrogen excretion determined at baseline and at the end of each study cycle. Total urine nitrogen excretion (E) content was determined using the micro-kjeldahl technique. Nitrogen intake (I) was estimated from protein intake that was determined from the analysis of the diet records over each of the respective urine collections. Conversion of protein intake to nitrogen is calculated as 16% of the total protein consumed for each collection period. Nitrogen balance is then calculated by subtracting urinary nitrogen from estimated nitrogen intake (nitrogen balance= I - E). Integumental nitrogen losses were estimated to be 200 mg per day and included in the nitrogen excretion calculations [13, 18].

Creatinine: To test for completeness of the 10hr and 24hr urine samples a creatinine test was done using an Urinary Creatinine Assay Kit (Cell Biolabs, Inc., San Diego, CA). Samples were analyzed in duplicate and total creatinine

(mg/day) was calculated using a standard curve and the formula creatinine (mg/dL)= ((corrected absorbance – y-intercept)/ slope) x sample dilution. Creatinine levels were then converted into milligrams per day. Normal creatinine levels have been found to be roughly between 500 and 2000 mg/day [1]

Statistical Analysis:

Group means for all criterion measures were calculated and differences determined using repeated measures ANOVA to evaluate the effects of supplementation. When differences were noted, post hoc analyses were done to determine specific effects between supplements. The Bonferroni method was used to correct for multiple comparisons and a paired student's t-test was performed. Statistical significance was set at $p< 0.05$. Statistical analysis was done using SPSS version 20.0 (SPSS, Inc., 2011; Chicago, IL).

Chapter IV

Results

Subject Characteristics

Age, height, weight, body fat percentage and maximal oxygen consumption (VO_{2max}) were determined at baseline. Weight was taken each time the subject returned to the lab to ensure subject was not gaining or losing weight. Baseline descriptive data are shown in table 4.1.

Table 4.1

Characteristics of Trained Male Runners at Baseline*

	Age	Height (cm)	Weight (kg)	Body Fat %	VO_{2max} (VO2/kg)
Baseline	22.0 ± 1.0	177.0 ± 2.3	68.0 ± 2.8	7.0 ± 0.9	66.0 ± 1.5

* mean ± SE

Macronutrient Intake

Descriptive data for macronutrient intake was obtained from diet records collected during each cycle of the investigation. The data for total energy intake and relative macronutrient intakes (carbohydrate, fat, protein) are shown in Table 4.2. On average, the macronutrient breakdown was 17% protein, 50% carbohydrate and 33% fat. A main treatment effect was found within subject contrast revealing an increase in protein percentage of the diet during Beef Jerky+ Powerade® compared to baseline ($F(1,7)= 9.16$, $p=.019$). Data is shown for baseline (BL), Powerade® (PA), Beef Jerky (BJ), and Beef Jerky+ Powerade® (BJPA).

Table 4.2				
Percent Energy, Protein, Carbohydrate and Fat Intake for Baseline, Powerade®, Beef Jerky, and Beef Jerky+ Powerade®*				
	Energy (kcal)	Protein (%)∞	Carbohydrate (%)	Fat (%)
Baseline	2576 ± 129	16 ± 2	51 ± 2	35 ± 2
Powerade®	2547 ± 116	17 ± 1	49 ± 3	33 ± 2
Beef Jerky	2442 ± 122	18 ± 1	51 ± 2	32 ± 2
Beef Jerky+ Powerade®	2471 ± 126	18 ± 1∞	51 ± 4	31 ± 2

* mean ± SE

∞ p≤ .05 compared to baseline

Macronutrient absolute values are shown in table 4.3. Subjects consumed the required 1.5 g/kg protein during each of the supplement cycles. There was a main treatment effect within subject contrasts revealing a reduction in grams of fat between baseline and Beef Jerky+ Powerade® ($F(1,7)= 9.47$, $p= .018$), as well as between Powerade® and Beef Jerky+ Powerade® ($p= .03$). A main treatment effect was seen within subject contrast for fat g/kg and a pairwise comparison for baseline and Beef Jerky+ Powerade® ($F(1,7)=8.70$, $p=.021$) and Powerade® and beef jerky+ Powerade® ($p=.028$) respectively.

Table 4.3						
Absolute Protein, Carbohydrate, and Fat Intake for Baseline, Powerade®, Beef Jerky, and Beef Jerky+ Powerade®*						
	Protein		Carbohydrate		Fat	
	g	g/kg	g	g/kg	g∞	g/kg∞
Baseline	97 ± 7	1.4 ± .1	326 ± 25	4.8 ± .4	98 ± 4	1.5 ± .1
Powerade®	105 ± 2	1.5 ± 0	333 ± 19	4.9 ± .3	95 ± 8	1.4 ± .1
Beef Jerky	111 ± 4	1.6 ± .1	315 ± 21	4.7 ± .4	89 ± 8	1.3 ± .2
Beef Jerky+ Powerade®	109 ± 3	1.6 ± .1	332 ± 21	4.9 ± .4	84 ± 7∞	1.2 ± .1∞

* mean ± SE

∞ p≤ .05 compared to baseline and Beef Jerky+ Powerade®

Protein Utilization

Nitrogen balance data (intake, excretion, and net) for baseline and all three supplement cycles are shown in table 4.4. Subjects were in positive balance at baseline and all treatments, but balance did not differ between treatments (p = .363). Figure 4.1 depicts nitrogen balance between baseline and supplements or between supplements (BL: 3.83 ± 1.71 vs. PA: 3.60 ± 1.90 vs. BJ: 3.96 ± 1.67 vs. BJPA: 7.88 ± 2.56) showing no significance.

Table 4.4			
Nitrogen Balance for Baseline, Powerade®, Beef Jerky, and Beef Jerky+ Powerade®*			
	Nitrogen Intake (g)	Nitrogen Excretion (g)	Nitrogen Balance (g)
Baseline	16.00 ± 2.47	12.17 ± 1.85	3.83 ± 1.71
Powerade®	17.10 ± 1.43	13.50 ± 1.69	3.60 ± 1.90
Beef Jerky	16.18 ± 1.75	12.22 ± 1.85	3.96 ± 1.67
Beef Jerky+ Powerade®	18.58 ± 1.90	10.70 ± 2.30	7.88 ± 2.56

* mean ± SE

Figure 4.1. Nitrogen Balance for Baseline, Powerade®, Beef Jerky, and Beef Jerky+ Powerade®. Means ± SE.

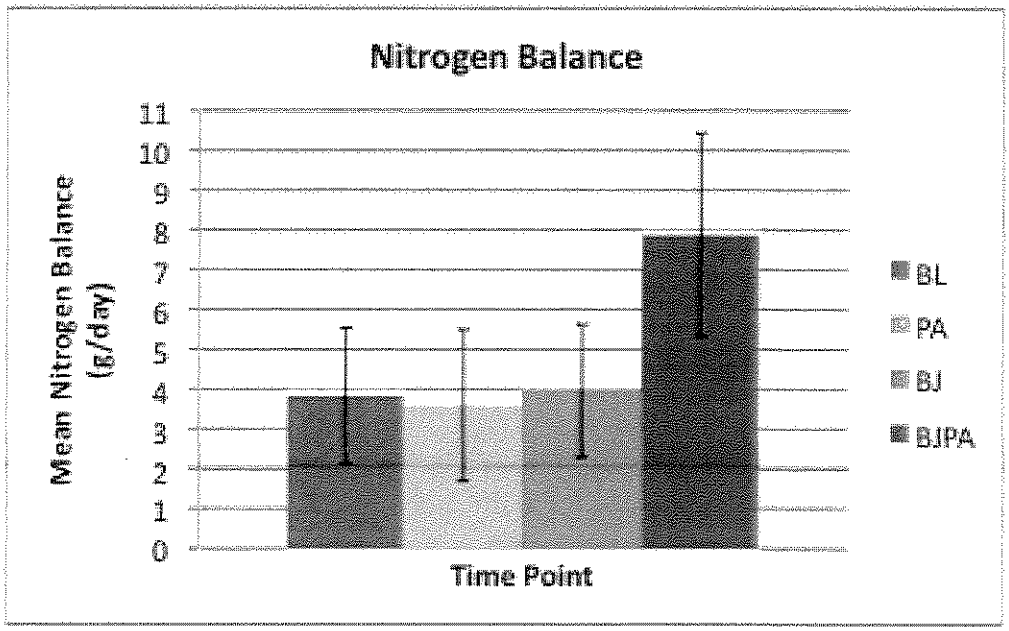


Table 4.5 shows protein turnover data for nitrogen flux (Q), protein synthesis (PS), protein breakdown (PB), and net protein balance [(NET)=PS-PB]. No significant differences were found in nitrogen flux between baseline and supplements or between supplements (BL: 1.71 ± 0.13 vs. PA: 1.73 ± 0.26 vs. BJ: 1.89 ± 0.20 vs. BJPA: 1.77 ± 0.25, p = .873) (Figure 4.2). Figure 4.3 (a-d)

shows PS, PB and NET for baseline, Powerade®, beef jerky, and beef jerky+ Powerade®. There was no significance in protein synthesis between baseline and supplements or between supplements (BL: 9.45 ± 0.80 vs. PA: 9.81 ± 1.57 vs. BJ: 10.66 ± 1.16 vs. BJPA: 9.66 ± 1.62, p = .837). Protein breakdown was also not significant between baseline and supplements and between supplements (BL: 10.00 ± 0.76 vs. PA: 9.39 ± 1.49 vs. BJ: 10.33 ± 1.26 vs. BJPA: 9.91 ± 1.41, p = .905). Net protein balance was not different between baseline and treatments or between treatments (BL: −.55 ± .27 vs. PA: .43 ± .37 vs. BJ: .33 ± .33 vs. BJPA: −.25 ± .50, p = .241).

Table 4.5

Nitrogen Flux (Q), Protein Synthesis (PS), Protein Breakdown (PB), and Net Protein Balance (NET) for Baseline, Powerade®, Beef Jerky, and Beef Jerky+ Powerade®*

	Q (g/kg/day)	PS (g/kg/day)	PB (g/kg/day)	NET (g/kg/day)
Baseline	1.71 ± 0.13	9.45 ± 0.80	10.00 ± 0.76	−.55 ± 0.27
Powerade ®	1.73 ± 0.26	9.81 ± 1.57	9.39 ± 1.49	.43 ± 0.37
Beef Jerky	1.89 ± 0.20	10.66 ± 1.16	10.33 ± 1.26	.33 ± 0.33
Beef Jerky+ Powerade ®	1.77 ± 0.25	9.66 ± 1.62	9.91 ± 1.41	−.25 ± 0.50

* mean ± SE

Figure 4.2. Nitrogen Flux for Baseline, Powerade®, Beef Jerky, and Beef Jerky+ Powerade®. Means ± SE.

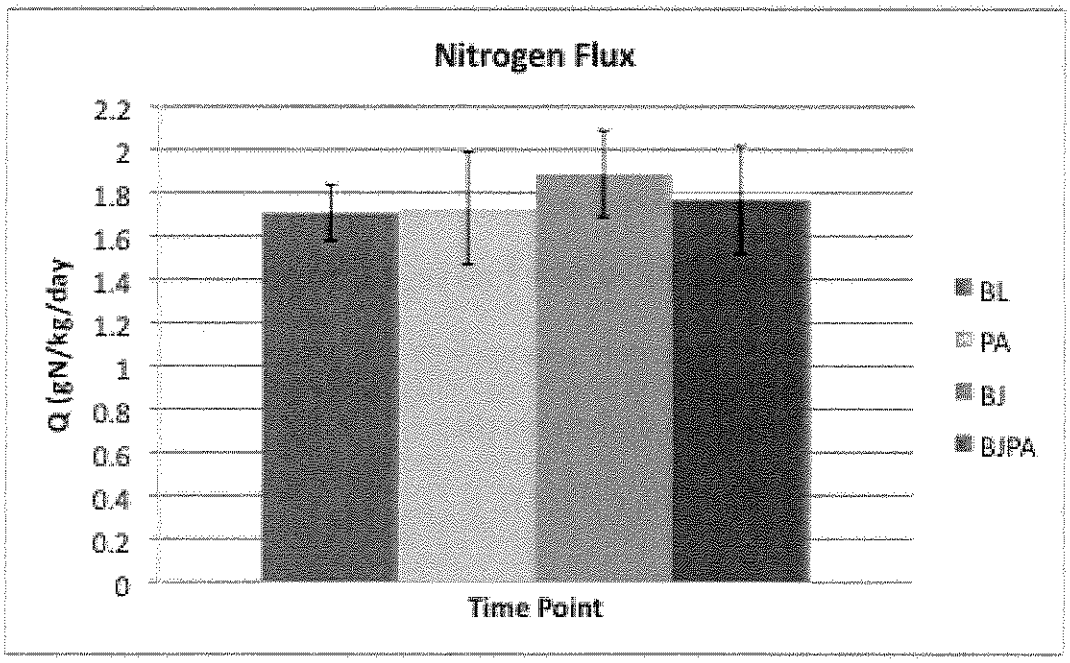
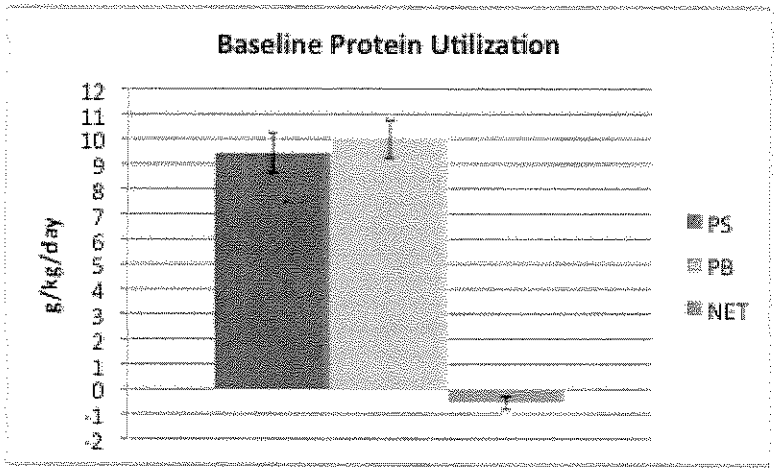
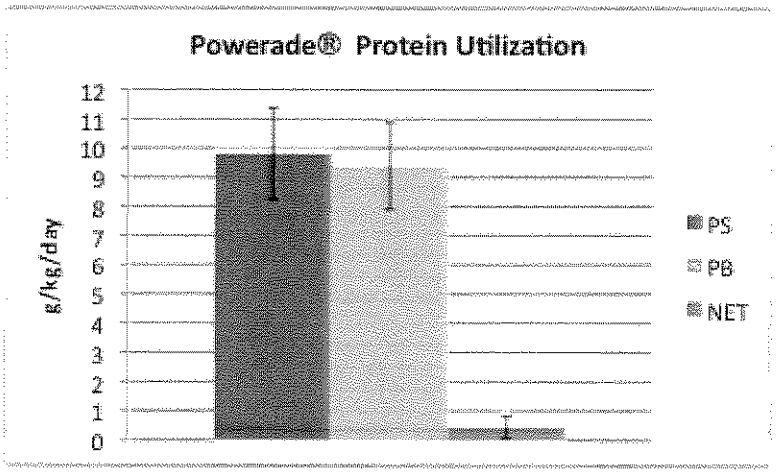


Figure 4.3 (a-d). Protein Utilization for Baseline, Powerade®, Beef Jerky, and Beef Jerky+ Powerade®. Means ± SE.

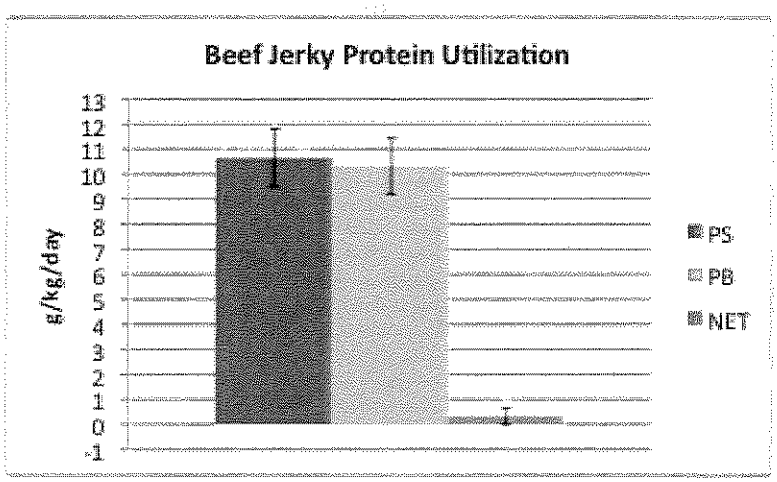
a.



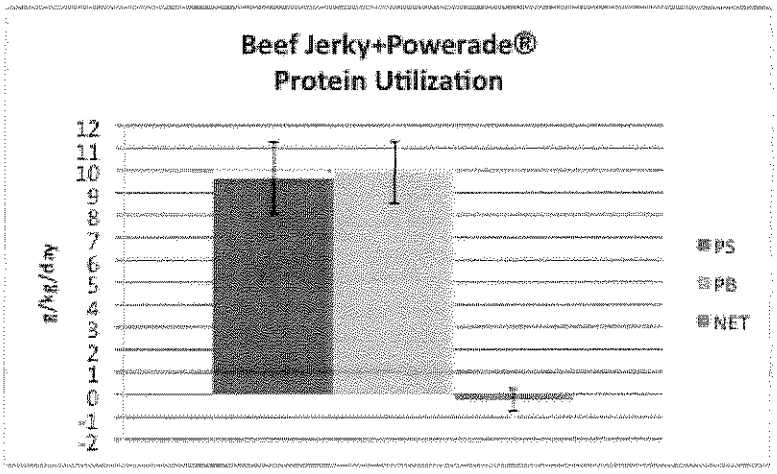
b.



c.


Beef Jerky Protein Utilization
g/kg/day
13
12
11
10
9
8
7
6
5
4
3
2
1
0
-1
PS
PB
NET

d.


Beef Jerky+Powerade®
Protein Utilization
g/kg/day
12
11
10
9
8
7
6
5
4
3
2
1
0
-1
PS
PB
NET

Creatinine:

Creatinine levels for all three cycles and baseline during the 10 and 24 hour urine collections are shown in table 4.6. While the mean for each group was within normal ranges there was some discrepancy with some individual 10 hour urine collections that were far below expected physiological levels.

Table 4.6

Ten Hour and Twenty-four Hour Creatinine Levels for Baseline, Powerade®, Beef Jerky, and Beef Jerky+ Powerade®*

	10 hour Creatinine (mg/d)	24 hour Creatinine (mg/d)
Baseline	357.25 ± 110.58	1165.88 ± 224.79
Powerade®	453.89 ± 153.99	1220.23 ± 120.11
Beef Jerky	457.60 ± 172.71	1272.10 ± 303.12
Beef Jerky+ Powerade®	453.28 ± 115.79	1134.11 ± 284.20

* mean ± SE

Chapter V

Discussion

This chapter discusses findings from the present investigation in the context of existing scientific literature as well as potential applications to the field of nutrition for recovery from exercise. In addition, limitations of the study are addressed. The significance of this work is presented and recommendations for future research are considered. While much data has been collected on protein turnover in strength athletes, comparatively much less has been collected on protein turnover in trained endurance athletes. The primary objective of the present investigation was to evaluate protein turnover and balance in response to three supplementation periods in trained male runners.

This study features novel work, in that it is the first study to look at beef jerky as a recovery supplement. The major finding of the present investigation was that while no significant differences were found in protein utilization or nitrogen balance between any of the supplement periods. Protein utilization was slightly positive for beef jerky and Powerade®, but was slightly negative for Beef Jerky+Powerade® although not significant. Nitrogen balance on the other hand was also not significantly different though it appeared to be increased to a greater extent in the Beef Jerky+Powerade® supplementation period. No significant changes in utilization are associated with no change in protein intake although there was a significant inverse relationship between fat intake and protein intake. This shows when consuming a diet of 1.5g/kg/d protein, taking a high quality protein supplement or carbohydrate-electrolyte beverage post

exercise, will not significantly impact protein utilization or significantly increase nitrogen balance.

Tarnopolsky et al. [60] showed endurance runners require more protein intake than both sedentary individuals and body builders to reach nitrogen balance. Their study showed endurance athletes need a protein intake of roughly 1.37 g/kg/day for nitrogen balance to occur in regularly training endurance athletes. Therefore, because our subjects consumed 1.5 g/kg/day protein, subjects were in positive nitrogen balance during each supplement period. Subjects in the present investigation consumed adequate amounts of calories for weight maintenance, and fat for energy requirements based on the perspective of the American Dietetics Association (ADA) [53]. However, carbohydrate intake was below levels suggested by the ADA [53]. The dietary protein intake must have contributing significantly to the overall net balance more than that of the supplements alone.

Endurance exercise effects protein turnover and net balance by increasing protein breakdown creating a negative balance. Pikosky et al. [50] found FSR increased but NET decreased in previously unfit runners following a four-week training program and a diet containing .8 g/kg/d protein. Carraro et al. [16] found similar findings but noted an increase in breakdown of 85% during 4 hours of recovery from endurance exercise at 40% VO_{2max} . This increase in breakdown was also followed by an increase in synthesis over the control (0.0821 ± 0.0006 v.s. 0.0654 ± 0.012%/hour) resulting in a decrease in net balance. These findings indicated the potential role exercise could play in decreasing net balance and

although the population in the current study differs from Pikosky et al. [50] and Carraro et al. [16], it could be possible that net balance would be even lower given the intensity and training status of our trained runners who run 6 days and over 30 miles per week.

Considering work done by Bolster et al. [8]and Pikosky et al. [49]it is possible that despite an increase in nitrogen balance, nitrogen flux, protein synthesis and protein breakdown can decrease. Bolster et al. [8]studied physically active children who were given a constant energy and protein intake and had been put on an aerobic exercise program, five days a week for a total of six weeks and found that while there was a significant increase in nitrogen balance from PRE to POST, nitrogen flux significantly decreased along with decreases in protein synthesis and protein breakdown. Pikosky et al. [49]also studied children who participated in a supervised resistance exercise program, two times per week for six weeks. It was found that although energy and protein intake remained constant throughout the study, significant decreases were observed in nitrogen flux, protein synthesis and protein breakdown following the six week training program. While the population differs from that of the current study, although the runners in the present investigation were highly trained, the increase in stress from the structured runs at 70% VO_{2max} and the timing of the 24 hour urine collection following the sixty minute run could have had elicited similar physiological responses in the current subjects as the aerobic training and resistance training did on the children. Recovery from the run might have increased energy needs

and energy intake might not have been increased enough given the increased expenditure to up regulate nitrogen flux and promote a more positive net balance.

Levenhagen et al. [35] found protein ingestion following a 60 minute bout of cycling at 60% VO_{2max} increased essential amino acid availability 33% and increased leg and whole body protein synthesis 6 fold and 15% respectively. This resulted in a net uptake of essential amino acids and net whole body and leg protein gain. When supplements were matched for energy with no protein, circulating amino acids uptake was no different when no supplement was taken. Overall when no supplement was provided there was a net loss in leg and whole body protein and even despite energy intake from a carbohydrate/fat supplement, there were still net losses in the leg and whole body protein. However, when 10g of protein was added to the carbohydrate/fat supplement, amino acid concentrations rose stimulating the fractional extraction and uptake of amino acids by the leg enhancing protein synthesis and overall leg and whole body protein accretion. The addition of protein following endurance exercise will enhance synthesis by allowing more amino acids to be available for synthesis increasing balance. More specifically, the leucine contained in the protein will have a direct effect on the protein synthesis pathway releasing the inhibition of the initiation translation factors 4E, 4G and S6 through the activation of the mTOR pathway [45].

The role of insulin in net balance cannot be overlooked. Biolo et al. [6] found a significant effect of insulin on protein degradation. Following a heavy resistance training workout, subjects were infused with insulin into the femoral

artery and tested for the subsequent 3 hours and FSR and FBR were determined from stable isotope tracers. The infused insulin increased protein synthesis at rest before exercise but showed no effect following exercise. On the other hand, insulin showed no effect on breakdown before exercise but significantly decreased protein breakdown following exercise. This supports our findings that show the lowest rate of protein breakdown came from the Powerade® cycle and the second lowest rate was in the beef jerky+ Powerade® cycle.

Borsheim et al. [9] studied the effects of 100g of maltodextrin carbohydrate (CHO) ingestion alone against a placebo (PLA) on net muscle protein balance following resistance exercise. Following a resistance-training workout of 10 sets of 8 reps at 80% 1RM subjects consumed either CHO or PLA 1 hour following exercise. Results showed an increase in net balance in the CHO group although still not positive balance. Protein supplements have been widely used and accepted for increasing net balance through increasing amino acid availability for protein synthesis and decreasing protein breakdown. Interestingly, results from the present investigation showed a positive balance for both Powerade® and beef jerky but was slightly negative during the beef jerky+Powerade® cycle.

Research on protein-carbohydrate recovery snacks has also shown great results for protein turnover. Howarth et al. [31] tested six active men who cycled a standard two hour protocol and randomly received a protein-carbohydrate (PRO-CHO), low carbohydrate (L-CHO) or high carbohydrate (H-CHO) drink and were tested for the first three hours following exercise. Results obtained at 0 and four hours showed FSR was higher for the protein-carbohydrate drink than either the

low or high carbohydrate drinks. In addition whole body net balance was only positive during the PRO-CHO drink, which was mainly attributable to a decreased protein breakdown. Interestingly, our results were similar in that they showed a decreased protein breakdown but were different in that of our three supplement cycles, only the beef jerky+ Powerade® cycle was negative although it wasn't significant. Given the similarity of decreased protein breakdown, it would be hypothesized that the difference in results comes from a lower FSR in the present investigation.

Recent published research from our lab shows the effects of fat free chocolate milk on protein balance which provides all essential amino acids as well as carbohydrates similar to that of our supplements. Lunn et al. [36] studied male runners who consumed either fat free chocolate milk (MILK) or a isocaloric non-nitrogenous carbohydrate control beverage (CON) following a 45 minutes run at 65% $VO_{2max.}$ Results show MILK supplementation resulted in higher FSR and less breakdown than that of CON. Lunn et al. concluded MILK consumption following endurance exercise added unique benefits for FSR and protein turnover than that of carbohydrate alone.

Other studies have similarly tested protein-carbohydrate drinks for recovery as amino acid-carbohydrate mixtures. Results were similar to those of others having significantly increased rates of synthesis while also having lower rates of breakdown although not significant [51]. Results from the present investigation differed possibly due to the difference in study design and procedure. The present investigation involved trained male runners who ran at a

higher intensity. In addition, studies used different tracer methods to test whole body net balance over a longer period of time. Other studies used a different population for their endurance protocol or tested net balance following resistance exercise. In either case investigators were interested in changes immediately post exercise. While the tracer method used in the present investigation is considered adequate for testing net balance, it cannot quantify net balance and FSR immediately following exercise. Therefore it is hard to directly compare results of the two studies with the present work but the differences do raise questions about net balance over a longer period of time than just a small window following exercise. These differences in study design could account for the differences in findings.

The results from the present investigation, although similar to those found in other studies, are different in that of the three supplement cycles, only the beef jerky+ Powerade® had a negative balance. Interestingly however beef jerky+ Powerade® also had the highest overall nitrogen balance, and although differences were not significant beef jerky+ Powerade® showed higher levels of nitrogen balance. Since results show a slightly negative net balance for beef jerky+ Powerade® the higher nitrogen balance could be due to the higher levels of overall protein received in the diet. Although this amount was not significant the modes increase may have contributed to the difference in nitrogen balance results.

Summary and Conclusions

The present investigation is the first study to look at protein utilization in response to ingestion of beef jerky and a carbohydrate-electrolyte beverage following endurance exercise in trained male endurance runners. This study is unique in documenting of the effects of consuming beef jerky as a recovery supplement given its high leucine content and also in conjunction and comparison with a standard recovery beverage. Although no differences in net balance between cycles was noted, the beef jerky+ Powerade® treatment was the only associated with a negative balance. Interestingly, the cycles which included Powerade® tended to produce the least protein breakdown and the cycles with the beef jerky tended to produce the greatest protein synthesis. These observations are consistent with those of previous investigations [6, 35]. Given the subjects were required to consume 1.5 g/kg/day it is not surprising all cycles resulted in positive nitrogen balance but beef jerky+ Powerade® tended to have higher nitrogen balance due to increased synthesis and decreased breakdown compared to other cycles. Therefore, the present investigation is consistent with previous research focused on protein carbohydrate recovery snacks. Although the beef jerky+ Powerade® cycle was expected to have the highest net balance, the lack of a documented difference, as well as the noted negative balance could be due to small sample size and variances which resulted from the combination of methods specific to whole body protein utilization and inter-individual differences.

Limitations

There are a number of limitations to the present investigation. These include the use of diet records to determine intake. The use of diet records has been shown to be an effective way to capture personal daily food and fluid consumption [3] however, in this case, diet records can be a limitation because of subject inability to accurately quantify the amount of food consumed or capture all ingredients used in preparation. Accurate assessment of nutrient intake especially protein is important for accurate results.

Another limitation is the completeness of urine collections and subject compliance to study protocols regarding administration of ^{15}N-glycine. Subjects were instructed on the administration of the isotope at home following dinner. As a result, data collection relies on the ability of subjects to properly administer the isotope and follow protocol. Furthermore, it is important for subjects to completely collect their urine both during the 10hr and 24hr collections. This protocol has been successfully used in previous investigations in our laboratory. Values obtained for urinary nitrogen and isotopic enrichments were within normal ranges and indicated that subjects adhered to study protocols.

Nitrogen balance methodology was used in combination with isotope methodology for determination of turnover. A number of assumptions exist in the nitrogen balance method, which presents a number of limitations to consider. The method of the present investigation uses estimates of nitrogen intake from protein sources recorded by subjects. Also, the excretory product used for analysis was urine as opposed to feces. These measures provide at best only an

estimate of nitrogen balance and of protein utilization in the body. As a result, the data is variable.

When considering statistical power, it seems that based on power calculations and previous research done using stable isotope tracers to calculate net protein balance, the study was powered appropriately to show differences in the ^{15}N-glycine method used. However, the calculated effect size for net balance for beef jerky was low to medium (4) indicating that the sample size might not have been large enough to show large differences between the 2 groups.

Finally, nitrogen losses were only calculated through urinary losses. Other nitrogen losses were only estimated and given the population of highly trained runners, integumental losses such as sweat may have been underestimated and could have impacted the final nitrogen balance results.

Significance

This investigation illustrates the ability to use a solid high quality protein food alone or in conjunction with a standard recovery beverage for recovery from sport. Even with aforementioned limitations, the work remains significant because it is unique in demonstrating that a high leucine, solid beef snack can be effectively used as a recovery snack following endurance exercise to promote net protein balance. When used with a standard recovery beverage beef jerky contributed to maintaining nitrogen balance. In addition, these results provide a base for further research on high leucine content solid foods to be used as recovery snacks post exercise.

Future Research Directions

Proper nutrition for recovery following any type of exercise is important. Given that most previous research has concentrated on nitrogen balance and protein turnover following resistance exercise, future investigations giving more attention to the physiological and physical consequences of endurance training and how to combat those effects with proper nutrition. Additional investigations are necessary to fully characterize the effects that daily and post exercise protein consumption have on endurance recovery. In addition, the ability to use whole food based products in a practical recovery protocol following an endurance exercise bout remains a worthy research initiative.

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